



POT/GB2004/004826



INVESTOR IN PEOPLE

The Patent Office  
Concept House  
Cardiff Road  
Newport

South Wales  
NP10 8QQ 07 JAN 2005

WIPO PCT

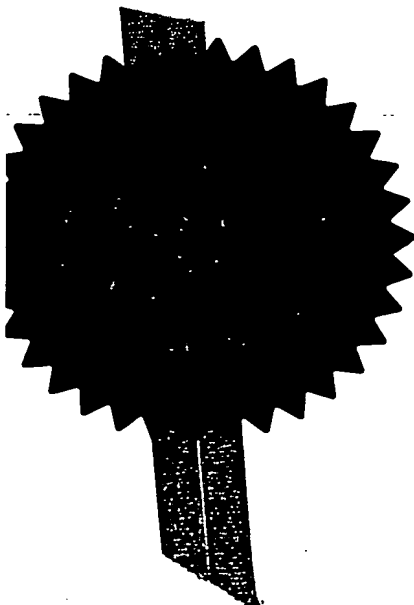
**PRIORITY  
DOCUMENT**  
SUBMITTED OR TRANSMITTED IN  
COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

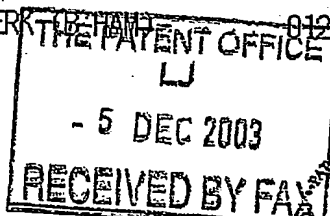


Signed

*Stephen Hordley*

Dated 15 December 2004

## Patents Form 1/77

Patents Act 1977  
(Rule 16)05DEC031E857460-1 D00355  
P01/7700 0.00#0328245.6

0328245.6

## Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road  
Newport  
South Wales  
NP10 8QQ

1. Your reference

W072537PGB

2. Patent application number

(The Patent Office will fill in this part)

05 DEC 2003

0328245.6

3. Full name, address and postcode of the or of each applicant (underline all surnames)

The University of Birmingham  
Research Park, Vincent Drive  
Birmingham B15 2SQ

08684318003

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

THERAPEUTIC USE OF BIOLOGICAL CELLS

5. Name of your agent (if you have one)

Marks &amp; Clerk

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Alpha Tower  
Suffolk Street Queensway  
Birmingham B1 1TT

Patents ADP number (if you know it)

18002 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number  
(if you know it)Date of filing  
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing  
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if)

- a) any applicant named in part 3 is not an inventor, or
  - b) there is an inventor who is not named as an applicant, or
  - c) any named applicant is a corporate body.
- See note (d))

## Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.  
Do not count copies of the same document

Continuation sheets of this form

Description 17

Claim(s)

Abstract

Drawing(s) 3 only

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11.

I/We request the grant of a patent on the basis of this application.

Signature

Date

5/12/03

12. Name and daytime telephone number of person to contact in the United Kingdom

David I Ward 0121 643 5881

## Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

## Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

### THERAPEUTIC USE OF BIOLOGICAL CELLS.

The present invention relates to a method of preparing biological cells for therapeutic use, as well as to methods of therapy utilising biological cells, and to medicaments comprising biological cells adapted for therapeutic use. The biological cells may preferably be stem cells or progenitor cells.

There are a growing number of contexts in which it is appreciated that biological cells may be utilised therapeutically. Perhaps the best known examples of therapies involving use of biological cells are:

- i) gene therapy, in which biological cells are modified to express therapeutic gene products, and the modified cells administered to a subject requiring therapy;
- ii) stem cell therapy, in which the multipotent properties of stem or progenitor cells are utilised to produce a therapeutic effect when the cells are administered to a subject requiring therapy (for instance by forming new tissue which may replace or augment damaged or abnormal tissues in the subject); and
- iii) immunotherapy, in which the immunological properties of biological cells are modified to produce a therapeutic effect, and the modified cells administered to a subject requiring therapy.

In each of the above examples the biological cells may be cells that are, or are derived from, cells isolated from the subject receiving therapy. In such cases the therapy is based on the use of autologous cells to achieve the therapeutic effect.

The advances in biological cell based therapies have created a need for techniques by which biological cells may be maintained *ex vivo* prior to their therapeutic use. The culture of stem or progenitor cells is particularly problematic using existing techniques. Current techniques make use of the activity of medium supplementation using serum, or cytokines such as interleukin-3 (IL-3), interleukin-11 (IL-11), stem cell factor (SCF) and Flt-3 ligand, to facilitate stem or progenitor cell propagation *ex vivo*. However, these existing techniques have many limitations, particularly in terms of their ability to maintain biological cells for sufficient time to allow their therapeutic adaptation, but without inducing cell maturation and differentiation. The ability to maintain stem or progenitor cells without causing maturation or

differentiation of the cells is of great importance in maximising the effectiveness of therapies utilizing biological cells for a number of reasons.

Firstly, in the case of stem cell therapy, the therapeutic effect of the adapted cells relies on their multipotent nature, which allows the cells to generate replacement tissue in the subject receiving treatment. If cells to be used for therapy undergo uncontrolled differentiation during culture the number of possible lineages into which they may develop, and hence their ultimate therapeutic potential, is reduced.

Secondly, in other therapeutic contexts it may be desirable to induce the controlled differentiation of biological cells into a required cell type prior to their administration to a subject requiring therapy. Such controlled differentiation allows the cells to be manipulated to produce a desired cell type having the greatest therapeutic value and aids the subsequent targeting of the adapted cells to tissues requiring the therapeutic activity. Existing techniques, in which differentiation occurs not at the direction of the practitioner but rather under the uncontrolled action of the "cocktail" of cytokines present in serum or cytokine supplemented media, prevent the purposeful generation of desired cell lineages and may lead to the production of mixed cell populations thereby reducing therapeutic effectiveness.

To date, none of the prior art techniques have been consistently successful enough to allow their widespread clinical use in biological cell-based therapies. There therefore remains a need to develop improved methods for preparing biological cells for therapeutic use, and improved methods of therapy utilizing biological cells.

According to a first aspect of the present invention there is provided a method of preparing a biological cell for therapeutic use, the method comprising the consecutive or concurrent steps of:

- i) culturing the biological cell in the presence of NM23 protein; and
- ii) adapting the biological cell for therapeutic use.

According to a second aspect of the present invention there is provided a method of therapy, the method comprising the consecutive or concurrent steps of:

- i) obtaining a biological cell;
- ii) culturing the biological cell in the presence of NM23 protein; and

iii) adapting the biological cell for therapeutic use and further comprising administering the adapted biological cell to a subject in need of such therapy.

The NM23 gene family comprises eight human members, designated NM23-H1 to NM23-H8, which encode eight different isoforms of NM23 protein. It has been reported that NM23-H1 protein and NM23-H2 protein share 88% homology, and that NM23-H3 protein has 70% homology with the NM23-H1 and NM23-H2 proteins. The present invention relates to the use of NM23 proteins generally, but preferably to the use of the NM23-H1 protein.

The amino acid sequence of NM23-H1 protein is shown as Sequence ID No. 1, and the sequence of cDNA encoding the protein is shown as Sequence ID No. 2. NM23 proteins suitable for use in accordance with the first, second and third aspects of the invention may share at least 60% homology with the sequence of Sequence ID No. 1, and may preferably share at least 70% homology. More preferably NM23 proteins in accordance with the invention may share at least 80% homology with Sequence ID No. 1, even more preferably at least 90% homology, and most preferably at least 95% homology.

It will be appreciated from the preceding paragraph that the present invention relates not only to wild-type isoforms of NM23 protein, but also to mutant forms, fragments, derivatives and analogues of such proteins that are able to exert the same biological effect as wild-type isoforms. Mutant proteins, protein fragments, derivatives and analogues suitable for use according to the invention may preferably be more biologically active, and/or more resistant to degradation, than wild-type proteins. Thus mutant proteins, protein fragments, protein derivatives and analogues may preferably have greater bioavailability to cultured cells, and indeed may have prolonged half-lives in culture conditions.

The present invention is based upon the inventors' discovery that NM23 proteins are able to function as both survival factors for cultured cells, and also as agents capable of preventing cell differentiation and maturation. As such NM23 proteins are of great benefit in culture of cells that are to be adapted for use in therapeutic applications, since cells cultured in the presence of NM23 retain the greatest possible therapeutic effectiveness.

Conventional prior art techniques rely on the use of "cocktails" of multiple cytokines to promote stem or progenitor cell expansion *ex vivo*. The cytokines are typically provided either as part of, or in addition to, serum supplementation. Commercially available media intended for use in the expansion of stem cell populations include factors such as interleukin-3 and interleukin-11 (IL-3 and IL-11), stem cell factor and Flt-3 ligand. The presence of such cytokines, while helping to promote cell division, causes maturation and differentiation of the cultured cells. This maturation is outside the control of the practitioner, and represents a major disadvantage, since it decreases the number of different cell lineages to which the cells may ultimately give rise and may prevent controlled differentiation of the cells into preferred cell types.

In contrast to prior art techniques, the inventors have found that supplementation of cell culture medium with NM23 protein promotes cell survival without differentiation, and without the need to provide serum or exogenous growth factors. Multipotent cells cultured in the presence of NM23 protein and adapted for therapeutic use are particularly beneficial in therapy since they retain their capability to give rise to a wide range of cell types (i.e. retain their multipotent characteristics). It is preferred that the cells are cultured in the presence of NM23 protein and media that are devoid of other cytokines or serum.

It is preferred that the NM23 protein may be provided as an extracellular protein, for instance as a supplement to cell (or tissue) culture media. The NM23 protein provided may be an exogenous or recombinant protein. If the NM23 protein is to be provided in cell culture medium it may preferably be provided at between 0.01 µg/ml and 1 mg/ml, more preferably at between 0.25 µg/ml and 500 µg/ml, even more preferably at between 0.5 µg/ml and 50 µg/ml, and most preferably at between 0.5 µg/ml and 5 µg/ml.

Suitably NM23 protein for provision as an extracellular protein to cultured cells may be produced by known techniques. For instance, the protein may be purified from naturally occurring sources of NM23 protein. Indeed, such naturally occurring sources of NM23 protein may be induced to express increased levels of the protein, which may then be purified using well-known conventional techniques. Alternatively cells that do not naturally express NM23 proteins may be induced to express such proteins. One suitable technique involves cellular expression of an NM23 protein/*his* construct. The expressed construct may subsequently be highly purified by virtue of the *his* "tag".

Alternatively, cells cultured in accordance with the invention may be induced to over-express NM23 protein. This effect may be achieved either by manipulating endogenous NM23 protein expression, or causing the cultured cells to express exogenous NM23 protein. Expression of exogenous NM23 protein may be induced by transformation of cells with well-known vectors into which cDNA encoding NM23 proteins may be inserted. It may be preferred that exogenous NM23 protein is expressed transiently by the cultured cell (for instance such that expression occurs only during *ex vivo* culture and ceases on administration of the cells to the subject requiring therapy).

It will be appreciated that the gene encoding the NM23 protein may be delivered to the biological cell without the gene being incorporated in a vector. For instance, the NM23 gene may be incorporated within a liposome or virus particle. Alternatively the "naked" DNA molecule may be inserted into the biological cell by a suitable means e.g. direct endocytotic uptake.

The exogenous NM23 gene (contained within a vector or otherwise) may be transferred to the biological cells by transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transfection with coated gold particles, liposomes containing the exogenous gene, and means of providing direct DNA uptake (e.g. endocytosis).

The methods of the present invention are suitable for use with a wide range of biological cell types, but are preferably to be used with stem or progenitor cells. For the purposes of the present invention stem cells are taken to comprise totipotent or pluripotent cells, and progenitor cells (or precursor cells) to comprise multipotent cells. Totipotent cells are those cells capable of giving rise to any type of differentiated cell found in an organism, whereas pluripotent cells are those cells capable of differentiating into several different final differentiated cell types. Multipotent cells are cells able to give rise to diverse cell types in response to appropriate environmental cues (such as action of soluble growth factors or the substrate on which the cell, or its progeny, is located).

The methods of the invention may utilise mesenchymal stem or progenitor cells. Such cells are capable of giving rise to fat cells, bone cells or cartilage cells, and may be harvested from blood, bone marrow, the spleen or adipose tissue according to techniques known to the art.

It will be appreciated that the precise nature of the biological cell selected for use in accordance with the invention may be determined on the basis of the therapeutic use to which the cell is to be put. For example, in the case where it is desired to effect therapy of the haematopoietic system it may be preferred to utilise a biological cell derived from the haematopoietic system. Similarly, where it is wished to effect therapy of the central nervous system (CNS) it will be preferred to utilise a biological cell derived from the CNS, and where it is wished to effect therapy of the epidermis it will be preferred to use an epidermal cell. Suitable protocols for the harvesting of biological cells for use in accordance with the invention will vary according to the source of the cells to be used. Cell harvest protocols are well known, and preferred protocols may be readily determined by those skilled in the art.

Preferably cells for use in the methods of the invention are collected from blood or bone marrow, most preferably from the peripheral blood. A most preferred method for collection of biological cells for use in accordance with the invention is described under 1.1 in the Example below.

Preferred culture conditions for use in accordance with the methods of the present invention may be determined with reference to the type of biological cell to be cultured. Consideration should be given both to the nature of the cell (e.g. stem or progenitor cell), to the source of the cell, and also to the manner in which the cell is to be adapted for therapeutic use. Suitable culture conditions are well known to those skilled in the art. Indeed it is a particularly advantageous feature of the invention that existing cell culture techniques may be readily modified for use in accordance with the first and second aspects of the invention by the incorporation of NM23 protein and exclusion of other supplementary cytokines or serum.

By way of example, it may be preferred to culture blood-derived cells in RPMI 1640 medium supplemented with NM23 protein, or to culture mesenchymal cells in NM23 protein-supplemented DMEM medium (media readily commercially available from supplier such as Gibco BRL). The inventors have found that it is particularly preferred to culture cells in accordance with the protocol provided at 1.2 in the Example below.

Step i) of the first aspect of the invention, or step ii) of the second aspect of the invention, may also involve *ex vivo* expansion of biological cell numbers. Such expansion may occur before, during, or after adaptation of the biological cells. The inclusion of NM23 protein (which the inventors have found acts as a survival factor for cultured cells) is particularly useful when expanding cell numbers *in vitro*.

Preferably the first aspect of the invention, and/or step ii) of the second aspect of the invention, may also comprise isolation of the biological cell from a human or non-human donor. Preferably the donor may be the subject requiring therapy.

It will be appreciated that cells formed according to the method of the first aspect of the invention may be utilised in the diagnosis of disorders *in utero*, and that methods according to both the first and second aspects of the invention may be used in the correction of such disorders.

The methods of the first and second aspects of the invention may be particularly useful in providing biological cells that have been adapted to express enzymes having therapeutic activity. Such cells may be used to replace or augment damaged, missing or abnormal cells of the subject.

There are many different ways in which biological cells may be adapted for therapeutic use in accordance with the invention. The nature of the adaptation to be made will be determined by the therapeutic use to which the adapted cell is to be put.

Typical adaptations that may be made to biological cells in accordance with the invention to enable their therapeutic use include:

- i) adaptations for use in gene therapy;
- ii) adaptations for use in stem cell therapy; and
- iii) adaptations for use in immunotherapy.

Examples of adaptations of biological cells for therapeutic use, in accordance with the invention, are considered in greater detail below. The following pages consider the use of biological cells that have been therapeutically adapted in accordance with the methods of the

invention for the treatment or prevention of a number of diseases and disorders. Except for where the context requires otherwise, it will be appreciated that the treatment or prevention of these diseases and disorders may be achieved using all embodiments of the second aspect of the invention, and is not limited to the specific adaptations considered below.

**1) Adaptations for use in gene therapy.**

Gene therapy is a technology by which genes or small DNA or RNA molecules may be transferred to cells, either to correct existing genetic defects or to prevent or treat genetically linked diseases. Such therapies represent a potentially very powerful method by which a wide range of medical disorders may be treated. The term "gene therapy" encompasses a number of different techniques able to achieve therapeutic effects in a number of ways, but in its broadest form may be thought of as the use of any recombinant genetic material (such as DNA, RNA or hybrid molecules) transferred to biological cells to achieve a therapeutic effect.

The present invention is particularly applicable to "*ex vivo*" gene therapy, in which gene transfer is carried out in culture and the adapted cells then administered to a subject requiring therapy. *Ex vivo* gene therapy is highly effective, allowing tight control of the manipulation of the cell as well as permanent integration of therapeutic genes into the adapted cells.

The use of an *ex vivo* gene therapy approach has a number of advantages over the use of *in vivo* gene transfer (in which a vector is injected directly into a patient to be treated). One of the clearest advantages of such an approach is the ability to perform extensive safety controls before the insertion of cells into the patient being treated. In addition higher levels of therapeutic gene expression may potentially be produced, as cells may be selected for production of high levels of therapeutic protein prior to their injection into the patient. The advantages of *ex vivo* gene therapy largely arise as a result of the potential for further therapeutic manipulation and testing that is afforded by the maintenance, and possible expansion, in culture of the therapeutically adapted cells. Unfortunately the application of *ex vivo* gene therapy has until now been limited by the lack of suitable culture techniques allowing growth and manipulation of the adapted cells.

Gene therapy may be designed to block the effect of deleterious faulty genes, such as those involved in Huntington's disease, by use of so called short, interfering RNAs (siRNAs). Gene therapy may also be used to repair errors in messenger RNA derived from defective

genes, and it is believed that this technique may have potential to treat various forms of cancer (including melanoma, leukaemia/lymphoma, prostate, ovarian and lung cancers) as well as other diseases such as cystic fibrosis and thalassaemia. Gene therapy has already been used in treatment of children with severe combined immunodeficiency disease, and it has been suggested that gene therapy may be useful in the treatment of neurodegenerative disorders such as Parkinson's disease. Other disorders that may be treated using gene therapy include haemophilia, familial hypercholesterolemia, Duchenne muscular dystrophy, AIDS and cardiovascular disorders.

Gene therapy also allows the development of DNA vaccines (which may be useful in the treatment of diseases such as malaria and AIDS, as well as endemic cancers such as Burkitt's lymphoma), and the production of replacement tissues and organs (expressing therapeutic genes) that may be used for transplantation.

Adaptation of a biological cell to make it suitable for gene therapy in accordance with the invention may typically comprise transformation of the cell to express a therapeutic product, typically a therapeutic gene product. Many suitable methods of adapting biological cells (such as stem cells and progenitor cells) in this manner are known to those skilled in the art. Typically cells may be transfected with a nucleic acid (herein referred to as a "therapeutic gene") that encodes a therapeutic product.

The therapeutic gene must be capable of being expressed by the adapted biological cells (preferably *in vitro* as well as when administered to a subject) yielding a product that, either directly or indirectly, has therapeutic activity. By "directly" we mean that the product of gene expression *per se* has therapeutic activity (for example a protein that replaces or augments abnormal protein expression in the subject). By "indirectly" we mean that the product of the therapeutic gene expression undergoes or mediates (e.g. as an enzyme) at least one further reaction to provide an agent having a therapeutic effect.

The therapeutic gene may be contained within a suitable vector to form a recombinant vector. The vector may for example be a plasmid, cosmid or phage. Such recombinant vectors are highly useful for transforming cells with exogenous genes.

Recombinant vectors may also include other functional elements. For instance, recombinant vectors may be designed such that the vector will autonomously replicate in the nucleus of the cell. In this case, elements that induce DNA replication may be required in the recombinant vector. Alternatively the recombinant vector may be designed such that the vector and recombinant DNA molecule integrates into the genome of a cell. In this case DNA sequences that favour targeted integration (e.g. by homologous recombination) are desirable. Recombinant vectors may also have DNA coding for genes that may be used as selectable markers in the cloning process.

The recombinant vector may also further comprise a promoter or regulator to control expression of the gene as required.

The therapeutic gene, or genes, may be inserted into a retroviral vector. Such vectors may advantageously fully integrate into the host genome. This results in long-term gene expression, with integrated genes passed onto daughter cells.

It is preferred that the therapeutic gene is inserted in an adenoviral vector. The use of adenoviral vectors avoids the risk of insertional mutagenesis as the vector remains episodic and is not integrated into the genome. In addition the adenoviral vector has good transduction ability in quiescent, non-dividing, highly differentiated cells, a property that may prove useful if it is desired to induce differentiation of the adapted cells prior to their administration to the subject.

It will be appreciated that the therapeutic gene, or genes, may be delivered to the biological cell without the gene being incorporated in a vector. For instance, the therapeutic gene, or genes, may be incorporated within a liposome or virus particle. Alternatively the "naked" DNA molecule may be inserted into the biological cell by a suitable means e.g. direct endocytotic uptake.

The therapeutic gene, or genes, (contained within a vector or otherwise) may be transferred to the biological cells by transfection, infection, microinjection, cell fusion, protoplast fusion or ballistic bombardment. For example, transfer may be by ballistic transfection with coated gold particles, liposomes containing the exogenous gene, and means of providing direct DNA uptake (e.g. endocytosis).

It may be preferred that cells cultured in accordance with the methods of the invention and adapted for use in gene therapy may be induced to differentiate and adopt a desirable cell lineage or phenotype prior to their administration to a patient. Such differentiation may be of benefit in ensuring correct targeting of the adapted cells within the patient (i.e. to ensure that the cells take up residence in a preferred tissue compartment to be treated). Cells that are to be induced to differentiate in accordance with this embodiment of the invention may be transformed with the therapeutic gene, or genes, before or after differentiation has taken place. Suitable factors to induce the required differentiation will be well known to those skilled in the art, and may be determined with reference to the lineage or phenotype that is desired for the cells to adopt.

**ii) Adaptations for use in stem cell therapy.**

Stem cell therapy represents a therapeutic method by which degenerative diseases (such as those caused by premature death or malfunction of cell types that the body is unable to replace) may be treated. It is hoped that addition of stem cells may help nucleate and promote the development of functional cells and/or tissues to replace those lost, thereby restoring normal healthy activity. Ultimately it may be possible to regenerate new functional tissues *ex vivo* which may then be administered to subjects requiring therapy.

The adaptation of biological cells for use in stem cell therapy may typically involve *ex vivo* expansion of stem cell or progenitor cell numbers in order to produce an increased stem cell population, the cells of which are suitable for administration to a subject requiring such therapy. In order to have therapeutic effectiveness, cells to be used in stem cell therapy (which may either be true stem cells or certain types of progenitor cells) must retain their ability to differentiate into multiple cell lineages when administered to a subject. Currently the application of stem cell therapy is limited by the lack of suitable methods by which stem cells may be propagated without undergoing differentiation and maturation.

Cells cultured in the presence of NM23 proteins are useful in methods of stem cell therapy since they promote stem cell survival in culture, and hence aid the expansion of stem cell numbers, but do not induce differentiation of the cultured cells.

It is believed that stem cell therapy may have wide applications across a broad range of diseases. For example stem cell therapy may be used in the treatment of blood disorders (such as leukaemia and sickle-cell anaemia), diseases of the brain and nervous systems (such as Parkinson's disease and Alzheimer's disease), musculo-skeletal disorders (such as muscular dystrophy, arthritis and osteoporosis), liver diseases (such as cirrhosis and hepatitis), spinal injuries, heart disease and diabetes.

Stem cell therapy may also be used to replace damaged tissue lost as a result of injury, trauma or cytotoxic insult. For example, such therapies may be used in neurodegenerative conditions, where CNS-derived stem cells may be utilised to replace or augment damaged somatic cells, such as those located in the brain or spinal cord. Stem cells may be used therapeutically in contexts in which the circulatory system has been injured, such as ischemic tissue damage after vascular occlusion. In such contexts suitable stems cells may be administered to cause formation of new blood vessels, or to replace other damaged tissues. Expanded populations of stem cells may also be utilised in conditions in which the liver has been damaged, in order to induce regeneration of the injured tissue.

### iii) Adaptations for use in immunotherapy.

Immunotherapy relates to a wide range of therapeutic methods in which immunological properties of biological cells are manipulated such that the cells exert a therapeutic effect when administered to a subject requiring therapy. Thus immunotherapy-based adaptations in accordance with the present invention comprise any adaptation of biological cells that render the cells suitable for use in immunotherapy-based methods.

Stem and progenitor cells culture in the presence of NM23 proteins may be adapted such that their immunological properties are manipulated while maintaining the multipotent character of such cells. For example such a manipulation may include the *ex vivo* adaptation of cells to take on the properties of antigen presenting cells (APCs). Cells having APC characteristics may then be further manipulated in order to express antigens associated with microbes or cells that it is desired to eliminate from the subject. For example, biological cells may be manipulated *ex vivo* to present antigens characteristic of cancer cells. Upon administration of the adapted cells to a subject these adapted cells (having APC characteristics) are able activate T cells thereby inducing the subject's adaptive immune response to target and kill cancer cells present.

A method of therapy according to the second aspect of the invention may utilise biological cells that have undergone such immunotherapy-based adaptations as a monotherapy in the treatment of cancer (i.e. use of the biological cell-based therapy alone) or may be used in combination with, or as an adjunct to, other cancer therapies known in the art.

Administration of therapeutically adapted cells in accordance with the second aspect of the invention may typically be by introduction of the cells into the subject's body. Such administration may preferably be achieved by means of injection, implantation or inhalation. Preferred routes of administration may be readily determined with reference to the disease to be treated.

It will be appreciated that biological cells prepared and adapted in accordance with the invention are also suitable for use in the preparation and manufacture of medicaments. Therefore according to a third aspect of the invention there is provided the use of a biological cell, cultured in the presence NM 23 protein and adapted for therapeutic use, as a medicament. Medicaments in accordance with this aspect of the invention are suitable for use in the treatment of the diseases, disorders and injuries considered above.

Medicaments in accordance with the third aspect of the invention may be formulated according to protocols well known in the art. Suitable formulations may be determined based on the preferred route by which the medicament is to be administered. Preferably medicaments according to the invention may be prepared in forms suitable for administration by inhalation, by injection, or by implantation.

Preferably formulations for inhalation may preferably comprise biological cells provided in a suitable liquid carrier. Such a liquid carrier is preferably non-immunogenic, and may comprise a saline solution, cell culture medium, or distilled water. Formulations for injection may be as described above, or may also be provided in the form of a gel, which may preferably be capable of resolution by the body of the subject treated. Formulations suitable for implantation may take the forms described for injection or inhalation, and may also comprise biological cells provided in a scaffold or matrix capable of providing a foundation for new tissue development.

In both methods of therapy according to the second aspect of the invention, and in the use of medicaments according to the third aspect of the invention, a therapeutically effective amount of biological cells adapted for therapeutic use should be administered to the subject requiring therapy. A "therapeutically effective amount" is any amount of therapeutically adapted cells which, when administered to a subject suffering from a disease against which the therapeutically adapted cells are effective, causes reduction, remission, or regression of the disease. A "subject" may be a human being, or any other animal, particularly a domestic or agricultural mammal.

The invention will be illustrated further by the following Example, and with reference to the accompanying drawings, in which:

Figure 1 shows the amino acid sequence of NM23 H1 protein, and the nucleic acid sequence of cDNA encoding NM23 H1 protein;

Figure 2 compares cell numbers in cultures of acute myeloid leukemia (AML) cells grown in the presence of NM23 H1 protein, with or without additional cytokines; and

Figure 3 compares the results of AML cell culture experiments using cytokine free media with or without NM23 H1 protein supplementation.

**EXAMPLE 1****PROTOCOLS****1.1. Cell harvesting.**

AML blast cells were isolated from peripheral blood (provided after informed consent) using Ficoll Hypaque cell density centrifugation.

**1.2. Cell culture.**

After harvesting, the cells were cultured overnight in RPMI 1640 medium supplemented with a commercial serum replacement (ITS<sup>+</sup>) and containing human recombinant interleukin 3 (IL3) and stem cell factor (SCF) (both at 5ng/ml).

The next day cells were washed twice (RPMI 1640; no ITS<sup>+</sup> nor cytokines) counted and adjusted to  $1 \times 10^6$  cells /ml in RPMI 1640 supplemented with ITS<sup>+</sup> and with or without IL-3 and SCF. Cells were plated as 1 ml cultures (in 48 well plates) in the presence and absence of recombinant human Nm23 H1 (provided at concentrations up to a maximum of 2 $\mu$ M).

After 5 days cells were harvested and the number of viable cells /ml of culture determined by the use of a haemocytometer and phase-contrast microscopy. Data showing dose responses to Nm23 H1 are for a single AML sample and the data points are the mean  $\pm$  SE of quadruplicate cultures. Data for the third figure are for n=9 consecutive AML samples received into the lab and that fitted the criteria of cells having high viability (>90%) after the initial overnight culture in the presence of IL-3 and SCF. These experiments used media without cytokines and the presence and absence of 2 $\mu$ g/ml Nm 23 H1.

**1.3. Expression and purification of recombinant NM23 H1.**

cDNA encoding NM23 H1 was inserted into the pET15b plasmid (Novagen) which carries and N-terminal His tag.

The protein product of the plasmid (i.e. NM23 H1 with a N-terminal His tag) was expressed in E.coli BL-21 (DE3) strain. Expression was induced using isopropyl-beta-D-thiogalactopyranoside (IPTG) in accordance with standard protocols.

The recombinant protein was purified using a conventional Nickel ion-chelated NTA agarose system according to protocols provided (Ni-NTA His-Bind Kit, Novagen).

## **RESULTS**

### **1.4. Recombinant Nm23 H1 acts as a stem and progenitor cell survival factor and does not cause differentiation of cultured cells.**

The inventors tested whether recombinant NM23 H1 protein, produced and purified according to the method set out in 1.3 above, was able to promote the survival of primary acute myeloid leukemia AML cells (which provide an experimental model for stem or progenitor cells) without inducing their differentiation.

Figure 2 shows data obtained from single AML samples incubated for 5 days with increasing NM23 H1 concentrations either in the absence of supporting cytokines (left panel) or in the presence of interleukin-3 (IL-3) and stem cell factor (SCF) (right panel).

Cultures were seeded at day 0 with  $10 \times 10^5$  cells (dashed line). The data show that NM23 H1 improved cell survival in the absence of cytokines and improved overall proliferation in their presence. Furthermore the AML cells cultured in the absence of other cytokines did not undergo maturation or differentiation during the period for which they were cultured.

Figure 3 shows collective data from  $n=9$  AML samples cultured for five days in the presence or absence of  $2\mu\text{g/ml}$  Nm23 H1 (without supporting cytokines). The dashed line again defines the number of cells plated at day 0.

The data shown in Figure 3 illustrate that all AML samples cultured in the absence of NM23 H1 (nine out of nine cultures tested) suffered loss of cells over the five day test period. In contrast two thirds of the samples cultured in the presence of NM23 H1 (six out of nine cultures tested) did not suffer cell loss, and in these cultures cell numbers remained the same or increased.

## **CONCLUSIONS**

### **1.5.**

The results set out in this Example illustrate that, in an experimental model of stem or progenitor cell culture, populations of cells grown in the presence of NM23 protein (and

without the addition of other cytokines) are able undergo expansion and are not subject to differentiation or maturation.

These results illustrate the effectiveness of NM23 protein in the propagation of biological cells that may, before, during or after such culture, be adapted for therapeutic use.

1/3

1 mancetfla ikpdgvqrgl vgeilkrfeg kgfrlvglkf mqasedllke hyvdikdrpf  
61 faglvkymhs gpvvarvweg lrvvktgrvm lgetnpadsk pgtirgdfci qvgrniihgs  
121 dsvesaekel glwfhpeelv dytscaqnwl ye

**SEQUENCE ID NO. 1**

1 atggccaact gtgagcgtac cticattgcg atcaaaccag atgggggtcca gcgggggtctt  
61 gtgggagaga ttatcaagcg tttgagcag aaaggattcc gcttgttgg tctgaaatc  
121 atgcaagcti ccgaagatct tcicaaggaa cactacgttg acctgaagga ccgtccattc  
181 ttgccgggoc tggtgaaata catgcactca gggccggtag ttgccatggt ctgggagggg  
241 ctgaatgtgg tgaagacggg ccgagtcag ctccggggaga ccaaccctgc agactccaag  
301 cctgggacca tccgtggaga ctctgcata caagtggca ggaacattat acatggcagt  
361 gattctgtgg agagtgcaga gaaggagatc ggcttgtgtt ttcacctga ggaactggta  
421 gattacacga gctgtgctca gaactggatc tatgaatga

**SEQUENCE ID NO. 2****Figure 1**

2/3

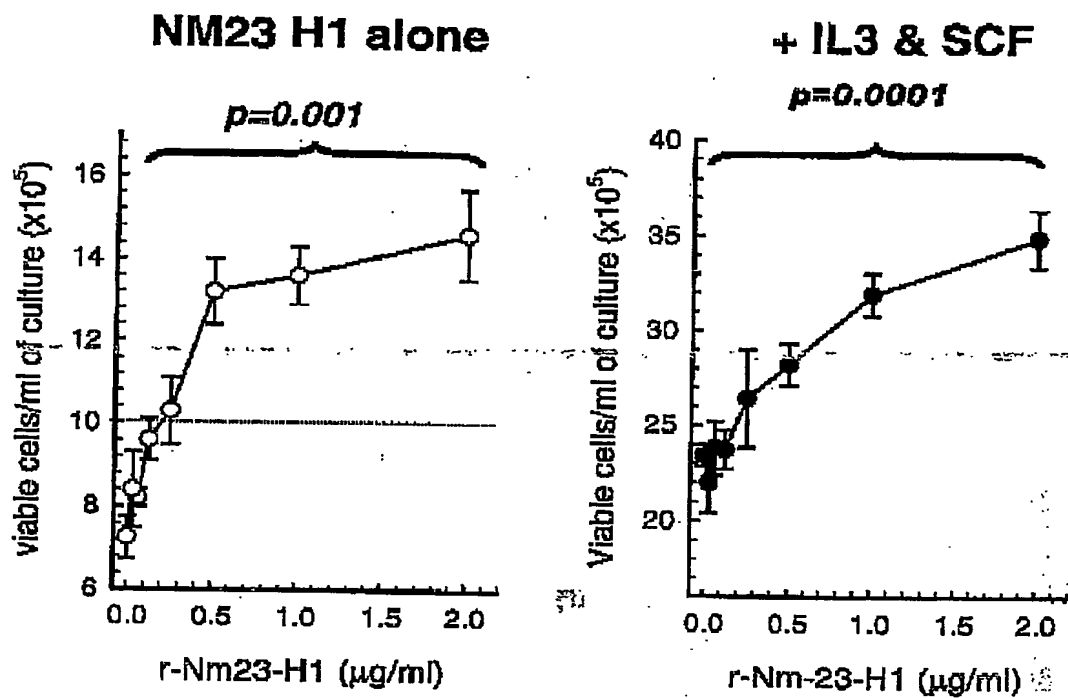
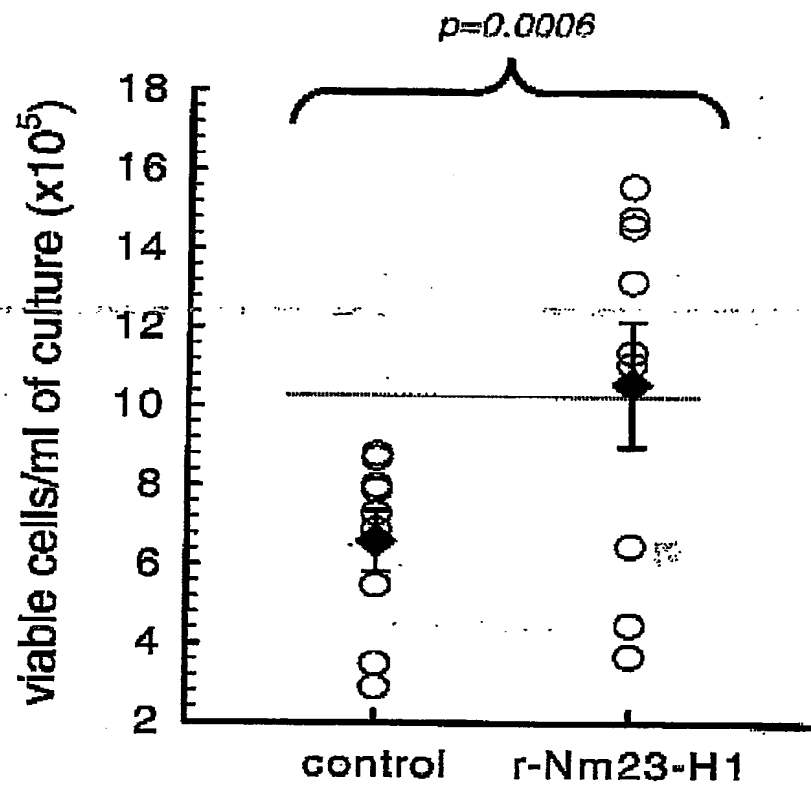


Figure 2

3/3

**NM23 H1 alone****Figure 3**

PCT/GB2004/004826



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**